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Manual

MutaPLEX® SC2 multi gene

Real-Time-RT-PCR-Kit

For the in vitro detection of RNA of SARS-CoV-2 (S gene, RdRP gene, E gene), extracted from biological specimens.

Valid from 2021-01-27

REF **KG193096**
KG1930-384
KG1930-768

Σ 96/384/768

-20°C

IVD

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Table of Contents

1 INTENDED USE

The MutaPLEX® SC2 multi gene Real-Time-RT-PCR kit is an assay for the detection of RNA of the pandemic coronavirus (SARS-CoV-2), extracted from biological specimens.

2 PATHOGEN INFORMATION

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The novel Coronavirus (SARS-CoV-2) is a new strain within the Sarbecoviruses that has been previously identified in humans and causes the pulmonary disease COVID-19 [1, 2, 3].

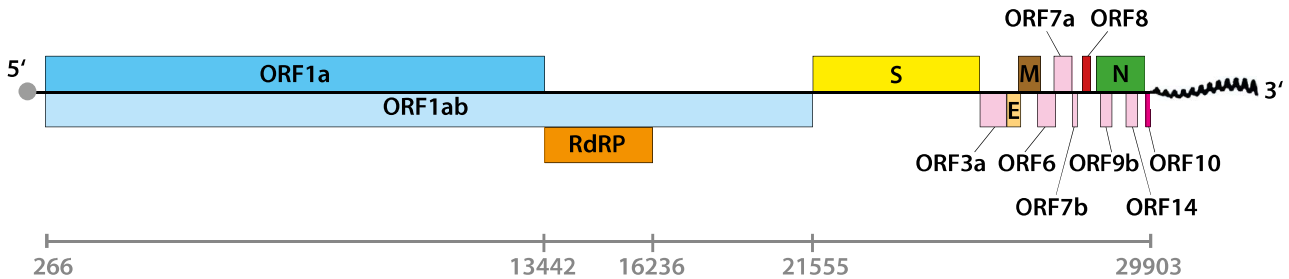
Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known Coronaviruses are circulating in animals that have not yet infected humans.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing.

3 PRINCIPLE OF THE TEST

The MutaPLEX® SC2 multi gene Real-Time-RT-PCR kit contains specific primers and dual-labelled probes for the amplification of RNA (cDNA) of SARS-CoV-2 (RdRP gene (RNA-dependent RNA polymerase) in the FAM channel, E gene (envelope protein) in the ROX channel and S gene (spike protein) in the Cy5 channel).



Schematic illustration: target gene region of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR kit.

Furthermore, MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit contains a Control RNA (Internal Process Control, IPC), which is added during RNA extraction and detected in the same reaction by a HEX-labelled probe. The Control RNA allows the

detection of RT-PCR inhibition and acts as control that the nucleic acid was isolated from the biological specimen.

4 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 (KG193096), 384 (KG1930-384), or 768 (KG1930-768) reactions, respectively.

Table 1: Components of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit .

Label	Lid Colour	Content		
		96	384	768
Reaction Mix	yellow	1 x 1325 µl	4 x 1325 µl	8 x 1325 µl
Enzyme	blue	1 x 19.2 µl	1 x 76.8 µl	2 x 76.8 µl
Positive Control (RdRP gene, E gene, S gene)	red	1 x 150 µl	1 x 300 µl	1 x 300 µl
Negative Control	green	1 x 150 µl	1 x 300 µl	1 x 300 µl
Control RNA	colourless	1 x 480 µl	2 x 960 µl	4 x 960 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- RNA isolation kit (e. g. MutaCLEAN® Mag RNA/DNA, KG1023 or KG1024)
- PCR grade water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortex
- Real time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® SC2 multi gene Real-Time-RT-PCR kit is shipped on dry ice or cool packs. All components must be stored at maximum -20 °C in the dark immediately after receipt. Up to 20 freeze and thaw cycles are possible. Do not use reagents after the date of expiry printed on the package.

For convenience, opened reagents can be stored at 2–8 °C for up to 6 months.

Protect kit components from direct sunlight during the complete test run.

7 WARNINGS AND PRECAUTIONS

- Stick to the protocol described in the instructions for use.
- The MutaPLEX® SC2 multi gene Real-Time-RT-PCR must be performed by qualified personnel only.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.

- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

8 SAMPLE MATERIAL

Starting material for MutaPLEX® Coronavirus (SARS-CoV-2) RT-PCR Kit is RNA isolated from biological specimens (e.g. swabs, sputum).

9 SAMPLE PREPARATION

Commercial kits for RNA isolation such as MutaCLEAN® Mag RNA/DNA (KG1023 or KG1024) are recommended.

Important: In addition to the samples, always run a water control in your extraction. Treat this water control analogous to a sample.

Comparing the amplification of the control RNA in the samples to the amplification of the internal control in the water control will give insights on possible inhibitions of the Real-Time-RT-PCR. Furthermore, possible contaminations during nucleic acid extraction will be detectable.

Please note chapter 10 "Control RNA".

If the Real-Time-RT-PCR is not performed immediately, store extracted nucleic acids according to the instructions given by the extraction kit's manufacturer.

10 CONTROL RNA

A control RNA is supplied as extraction control. This allows the user to control the RNA isolation procedure and to check for possible Real-Time-RT-PCR inhibition.

Add 5 µl control RNA per extraction (5 µl x (N+1)). Mix well. Perform the RNA isolation according to the manufacturer's instructions.

The control RNA must be added to the lysis buffer of the extraction kit.

11 REAL-TIME-RT-PCR

11.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-RT-PCR familiarise yourself with the real time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the RT-PCR set up.
- In every RT-PCR run, one positive control and one negative control should be included.
- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed and centrifuged very briefly.
- Due to the high viscosity of the enzyme (blue lid), prewarming at room temperature for 15 min is recommended.

11.2 Procedure

The control RNA was added during RNA extraction (see chapter 10 "Control RNA"). Prepare the master mix according to table 2.

The master mix contains all of the components needed for RT-PCR except the sample. Prepare a volume of master mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 2: Preparation of the master mix (control RNA was added during RNA extraction)

Volume per reaction	Volume master mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Real-Time-RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the real time PCR instrument / take an optical PCR reaction plate.
- Pipet **14 µl** of master mix into each optical PCR reaction tube.
- Add **6 µl** of the eluates from the RNA isolation (including the eluate of the water control), the respective positive control, and the negative control the corresponding optical PCR reaction tube / the optical PCR reaction plate (table 4).

- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the Real-Time-RT-PCR

Component	Volume
Master mix	14.0 µl
Sample	6.0 µl
Total volume	20.0 µl

11.3 Instrument settings

For the Real-Time-RT-PCR use the thermal profile shown in table 4.

Table 4: Real-Time-RT-PCR thermal profile

Description	Time	Temperature	No of cycles
Reverse Transcription	10 min	45 °C	1
Initial Denaturation	5 min	95 °C	1
Amplification of cDNA			45
Denaturation	10 s	95 °C	
Annealing and extension	40 s	60 °C	
	Aquisition at the end of this step		

Dependent on the real time instrument used, further instrument settings have to be adjusted according to table 5.

Table 5: Overview of the instrument settings required for the MutaPLEX® SC2 multi gene Real-Time-RT-PCR.

Real-Time-RT-PCR-instrument	Parameter	Detection channel	Notes
LightCycler 480II	RdRP gene Control RNA (IPC) E gene S gene	465–510 533–580 533–610 618–660	Colour Compensation Kit CC-1 (KG19-5-CC) required

Melt factor	Quant factor	Max integration time (s)
1	10	1
1	10	2
1	10	2
1	10	3

Real-Time-RT-PCR-instrument	Parameter	Detection channel	Notes	
Stratagene Mx3000P/ Mx3005P	RdRP gene Control RNA (IPC) E gene S gene	FAM HEX ROX Cy5	Gain 8 Gain 1 Gain 1 Gain 4	Reference Dye: None
ABI 7500	RdRP gene Control RNA (IPC) E gene S gene	FAM JOE ROX Cy5	Option Reference Dye ROX: NO	
AriaMx Bio-Rad CFX96	RdRP gene Control RNA (IPC) E gene S gene	FAM HEX ROX Cy5	Option Reference Dye ROX: NO	
Rotor-Gene Q, Rotor-Gene 3000 Rotor-Gene 6000	RdRP gene Control RNA (IPC) E gene S gene	Green Yellow Orange Red	Gain 5 Gain 5 Gain 5 Gain 5	
Mic qPCR Cycler	RdRP gene Control RNA (IPC) E gene S gene	Green Yellow Orange Red	Gain 8 Gain 10 Gain 10 Gain 10	

12 DATA ANALYSIS

The following results can occur (table 6):

Table 6: Interpretation of the results

Signal/C_T Values				Interpretation
FAM channel	**ROX channel**	**Cy5 channel**	**HEX channel**	
RdRP Gene	E gene	S gene	Control RNA (IPC)	
positive	**positive**	**positive**	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA.**
positive	**positive**	negative	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA.**

Signal/C_T Values				Interpretation
FAM channel	**ROX channel**	**Cy5 channel**	**HEX channel**	
RdRP Gene	E gene	S gene	Control RNA (IPC)	
positive[3]	negative	negative	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA.**
negative	**positive**	**positive**	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA.**
negative	**positive**[3]	negative	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA. or SARS-CoV-1 RNA[4].**
negative	negative	**positive**[3]	positive or negative[1]	**Positive result, the sample contains SARS-CoV-2 RNA.**
negative	negative	negative	< 34	**Negative result. The sample contains no RNA of SARS-CoV-2 and SARS-CoV-1[4].**
negative	negative	negative	negative or > 34[2]	**Caution!** The real time RT-PCR is either inhibited or errors occurred while RNA/DNA extraction.

1 A strong positive signal in the FAM, Cy5 or ROX channel can inhibit the IPC. In such cases the result for the Control RNA can be neglected.

2 In case of high CT values, the IPC should be compared to the water extraction control as described in the chapter 'Assay validation'.

3 The WHO Guidelines for the detection of SARS-CoV-2 (March 19, 2020) recommend the detection of two different targets in areas with no known SARS-CoV-2 circulation (Lit. [4]).

4 SARS-CoV-1 infections have not been reported since 2004 (Lit. [5]).

Figure 1, 2, 3 and **4** show examples for positive and negative real time RT-PCR results.

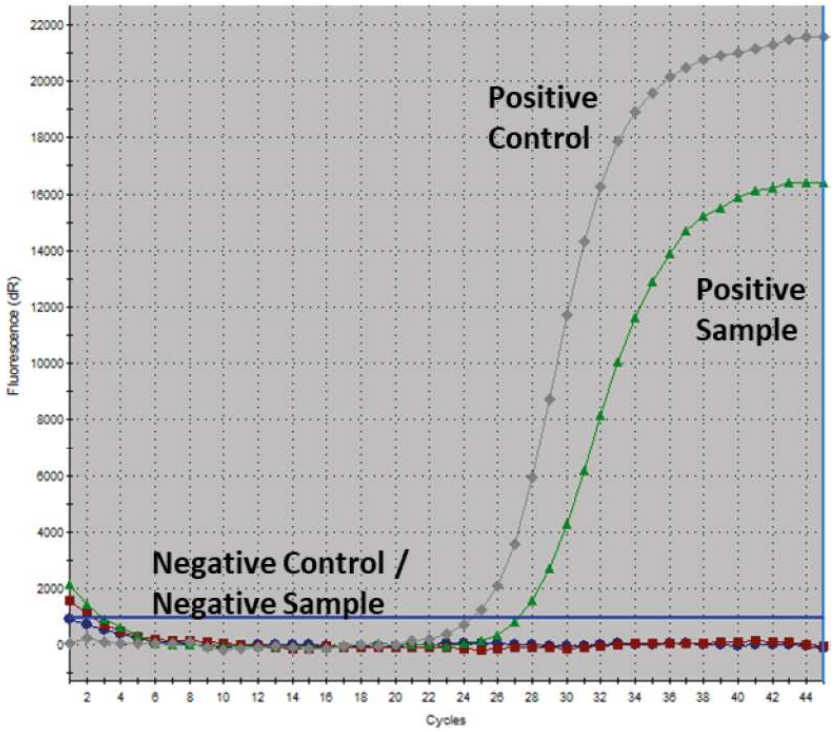


Figure 1: The positive sample shows pathogen specific amplification in the FAM channel (positive sample and Positive Control), whereas no fluorescence signal is detected in the negative sample or the Negative Control (Mx3005P qPCR System).

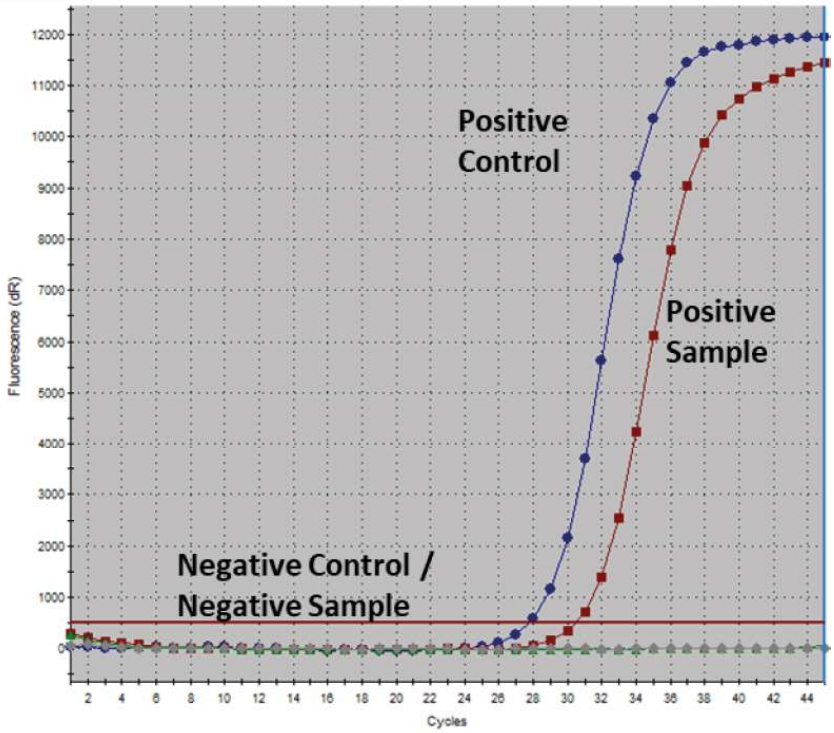


Figure 2: The positive sample shows pathogen specific amplification in the ROX channel (positive sample and Positive Control), whereas no fluorescence signal is detected in the negative sample and the Negative Control (Mx3005P qPCR System).

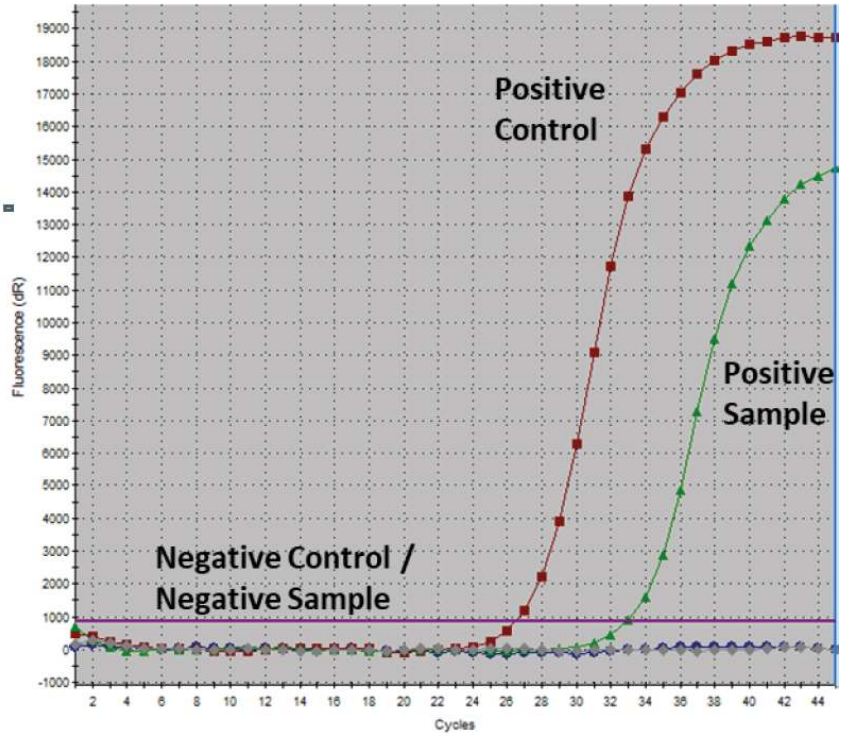


Figure 3: The positive sample shows pathogen specific amplification in the Cy5 channel (positive sample and Positive Control), whereas no fluorescence signal is detected in the negative sample and the Negative Control (Mx3005P qPCR System).

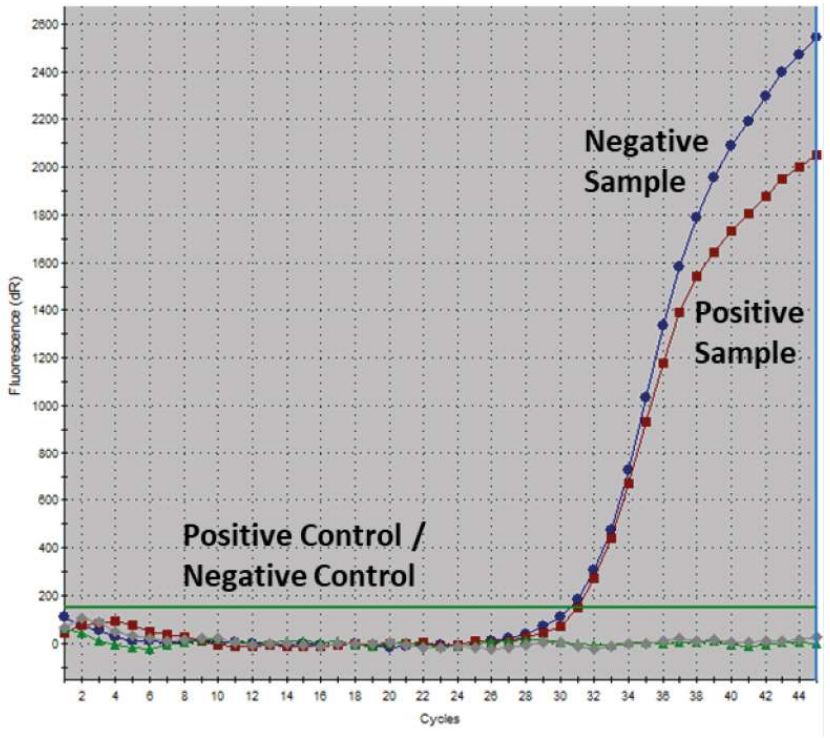


Figure 4: The positive sample and the negative sample show an amplification curve, whereas the Positive Control and the Negative Control don't show a signal in the Control RNA specific HEX channel (Mx3005P qPCR System).

13 ASSAY VALIDATION

Negative controls

The Negative Control must show no CT in the FAM, HEX, ROX and Cy5 channel.

Positive controls

All parameters in the Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5 and ROX. The Positive Control must fall below a CT of 30. The Positive Control includes in vitro transcripts of SARS-CoV-2 (RdRP gene, E gene and S gene).

Internal controls

The following values for the amplification of the internal control (IPC) are valid using Immundiagnostik nucleic acid extraction kits MutaCLEAN® Mag RNA/DNA or MutaCLEAN® Universal RNA/DNA.

The IPC must show a positive (i.e. exponential) amplification curve. The Control RNA (IPC) must fall below a C_T of 34. If the Control RNA is above C_T 34 this points to a purification problem or a strong positive sample that can inhibit the IPC. In the latter case, the assay is valid. It is recommended to perform the extraction of a water control in each run. The IPC in the water control must fall below a C_T of 34.

If other nucleic acid extraction kits are used, the customer must define own cut-offs. In this case the C_T value of the Control RNA in an eluate from a sample should not be delayed for more than 4 C_T in comparison to an eluate from an extracted water control.

14 LIMITATIONS OF THE METHOD

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.

- Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers and/or probes used in the kit may result in failure to detect the respective RNA.
- As with any diagnostic test, results of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

15 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-RT-PCR.

No fluorescence signal in the FAM and/or ROX and/or Cy5 channel of the positive control

The selected channel for analysis does not comply with the protocol

Select the FAM channel for analysis of the RdRP gene specific amplification, the ROX channel for analysis of the E gene specific amplification, the HEX channel for the amplification of the Control RNA and the Cy5 channel for the amplification of the S gene.

Incorrect preparation of the Master Mix

Make sure the enzyme is added to the master mix (chapter 11).

Incorrect configuration of the Real-Time-RT-PCR

Check your work steps and compare with chapter "Procedure".

The programming of the thermal profile is incorrect

Compare the thermal profile with the protocol (table 4 and table 5).

Incorrect storage conditions for one or more kit components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Weak or no signal of the Control RNA and simultaneous absence of a signal in the FAM and/or ROX and/or Cy5 channel.

Real-Time-RT-PCR conditions do not comply with the protocol

Check the Real-Time-RT-PCR conditions (chapter 11).

Real-Time-RT-PCR inhibited

Make sure that you use an appropriate isolation method (see "Sample preparation") and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffer of the isolation kit has been completely removed.

Sample material not sufficient

Make sure enough sample material has been applied to the extraction. Use an appropriate isolation method (see chapter "Sample preparation") and follow the manufacturer's instructions

RNA loss during isolation process

In case the control RNA was added before extraction, the lack of an amplification signal can indicate that the RNA isolation was not successful. Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.

Incorrect storage conditions for one or more components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter "Transport, storage and stability".

Detection of a fluorescence signal in the FAM and/or ROX and/or Cy5 and/or HEX channel of the negative control

Contamination during preparation of the RT-PCR

Repeat the Real-Time-RT-PCR in replicates. If the result is negative in the repetition, the contamination occured when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the positive control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the Real-Time-RT-PCR.

16 KIT PERFORMANCE

16.1 Analytical sensitivity

The limit of detection (LoD) of MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit was determined testing serial dilutions of synthetic RNA-fragments containing the specific gene target sequence on a QuantStudio 5 real time PCR instrument. The estimated LoD of MutaPLEX® SC2 multi gene real time RT-PCR Kit is ≤ 10 genome copies

per reaction for the SARS-CoV-2 RdRP gene and 1 genome copy per reaction for the SARS-CoV-2 E gene and the S gene.

16.2 Analytical specificity

The specificity of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit was evaluated with different other relevant viruses and bacteria found in clinical samples and basing on in silico analyses.

The results for the sample analysis are shown in table 7 and table 8, the result for the in silico analysis of the Primer and Probe binding sites is shown in table 9.

For in silico exclusivity testing, all Primers where used in BLAST analysis with exclusion of the specific PCR targets. Primers and Probes for SARS-CoV-2 E gene may detect SARS-CoV-1 as well, but since there is no report on SARS-CoV-1 cases since 2004, it is very unlikely to happen [6]. This is the only nontarget sequence detected in silico for potential amplification.

Table 7: AccuPlex™ SARS-CoV-2 Verification Panel.

	Result SARS-CoV-2	**Result SARS-CoV-2**	**Result SARS-CoV-2**
	RdRP gene	**E gene**	**S gene**
Member 1 100.000 copies/ml	**positive**	**positive**	**positive**
Member 2 10.000 copies/ml	**positive**	**positive**	**positive**
Member 3 1.000 copies/ml	**positive**	**positive**	**negative**
Member 4 5000 copies/ml RNase P	**negative**	**negative**	**negative**

Table 8: Eluted nucleic acid from bacterial and viral pathogens tested for the determination of the analytical specificity of MutaPLEX® SC2 multi gene real time RT-PCR Kit.

Eluates with known status	**Result SARS-CoV-2**	**Result SARS-CoV-2**	**Result SARS-CoV-2**
	RdRP gene	**E gene**	**S gene**
Parainfluenzavirus 1	negative	negative	negative
Parainfluenzavirus 2	negative	negative	negative

Eluates with known status	Result SARS-CoV-2	Result SARS-CoV-2	Result SARS-CoV-2
	RdRP gene	E gene	S gene
Parainfluenzavirus 3	negative	negative	negative
Parainfluenzavirus 4	negative	negative	negative
Metapneumovirus	negative	negative	negative
Adenovirus	negative	negative	negative
Rhinovirus	negative	negative	negative
Enterovirus	negative	negative	negative
Human Bocavirus	negative	negative	negative
Legionelle pneumophila	negative	negative	negative
Mycoplasma pneumophila	negative	negative	negative
Mycobacterium tuberculosis complex	negative	negative	negative
Bordetella pertussis	negative	negative	negative
Bordetella parapertussis	negative	negative	negative
Staphylococcus aureus	negative	negative	negative
MRSA	negative	negative	negative
MSSA	negative	negative	negative
Streptococcus spp.	negative	negative	negative
SARS-CoV-2	**positive**	**positive**	**positive**
HCoV-OC43	negative	negative	negative
HCoV-229E	negative	negative	negative
MERS-CoV	negative	negative	negative
Influenzavirus A H1N1	negative	negative	negative

Eluates with known status	Result SARS-CoV-2	Result SARS-CoV-2	Result SARS-CoV-2
	RdRP gene	E gene	S gene
Influenzavirus A H3N2	negative	negative	negative
Influenzavirus A H5N1	negative	negative	negative
Influenzavirus B	negative	negative	negative
Respiratory Syncytial Virus A	negative	negative	negative
Respiratory Syncytial Virus B	negative	negative	negative

Table 9: Inclusivity of the MutaPLEX® SC2 multi gene real time RT-PCR Kit primers and probes (*in silico* analysis).

1000 - 5000 whole genome sequences		Homology	Comment
SARS-CoV-2 RdRP gene	Forward primer	2313 sequences: 100%	7 sequences: 95% (1 mismatch)
	Reverse primer	2320 sequences: 100%	no mismatch
	Probe	2318 sequences: 100%	2 sequences: 95% (1 mismatch)
SARS-CoV-2 S gene	Forward primer	2315 sequences: 100%	5 sequences: 96% (1 mismatch)
	Reverse primer	2312 sequences: 100%	8 sequences: 96% (1 mismatch)
	Probe	2309 sequences: 100%	11 sequences: 95% (1 mismatch)
SARS-CoV-2 E gene	Forward primer	2319 sequences: 100%	5 sequences: 96% (1 mismatch)
	Reverse primer	2318 sequences: 100%	2 sequences: 95% (1 mismatch)
	Probe	2317 sequences: 100%	3 sequences: 96% (1 mismatch)

16.3 Clinical samples

Positive (36) and negative (171) confirmed samples (oral and nasal swabs) from the pandemic COVID-19 outbreak 2020 in Europe were tested.

The RNA was extracted by using the MutaCLEAN® Mag RNA/DNA (KG1023) extraction kit on a KingFisher Prime Duo Instrument.

The PCR experiments were performed on a QuantStudio 5 Cycler. The testing of the confirmed samples with MutaPLEX® SC2 multi gene real time RT-PCR Kit showed a sensitivity of 100% and a specificity of 100%. None of the samples were inhibited in the real time RT-PCR. For the validation of the respiraSC2 multi gene real time RT-PCR Kit the eluates of all samples were retested and showed the same results.

field samples (2020)	**SARS-CoV-2 Positive samples**	**SARS-CoV-2 Negative samples**
MutaPLEX® SC2 multi gene positive	36	0
MutaPLEX® SC2 multi gene negative	0	171
	Sensitivity [%]	**Specificity [%]**
	100	100

Additionally, samples from different ring trials were tested with the respiraSC2 multi gene real time RT-PCR.

ring trials (2020)	**SARS-CoV-2 Positive samples**	**SARS-CoV-2 Negative samples**
MutaPLEX® SC2 multi gene positive	11	0
MutaPLEX® SC2 multi gene negative	0	19
	Sensitivity [%]	**Specificity [%]**
	100	100

Detailed information is available at Immundiagnostik AG.

16.4 Linear range

The linear range of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit was evaluated by analysing logarithmic dilution series of in vitro transcripts (SARS-CoV-2 RdRP gene, S gene and E gene).

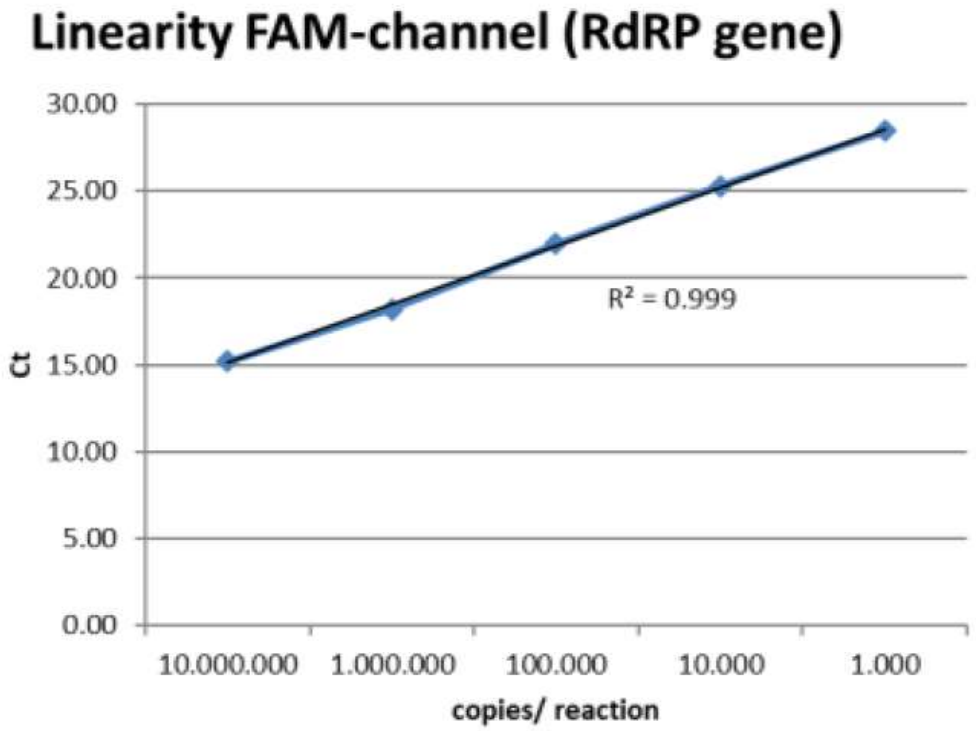


Figure 5: Determination of the linear range of MutaPLEX® SC2 multi gene Real-Time-RT-PCR in the FAM channel.

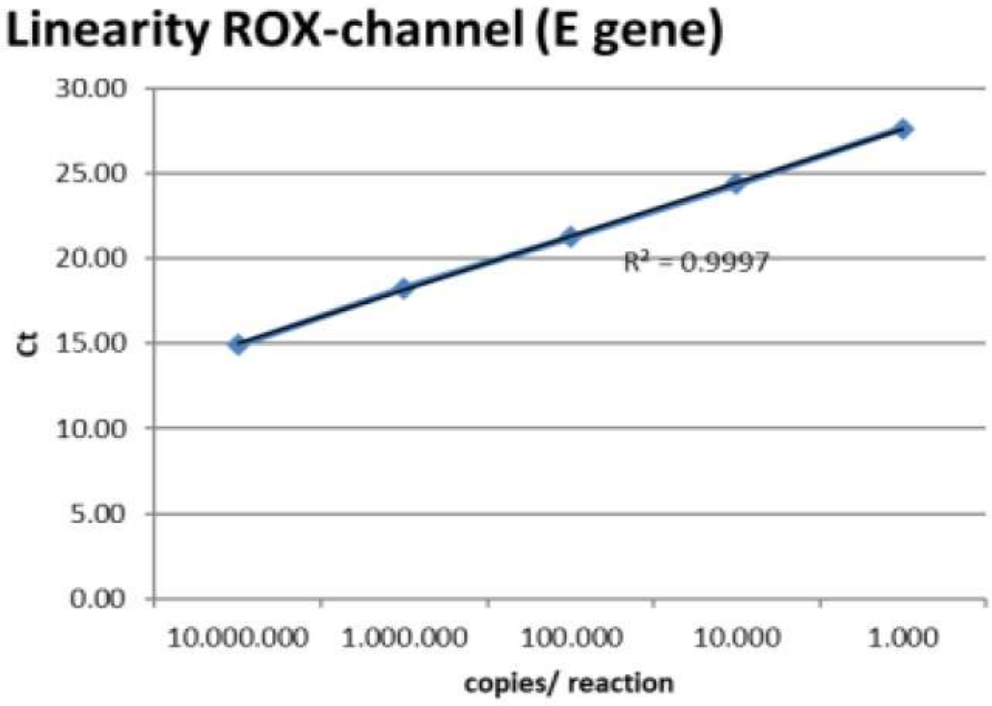


Figure 6: Determination of the linear range of MutaPLEX® SC2 multi gene Real-Time-RT-PCR in the ROX channel.

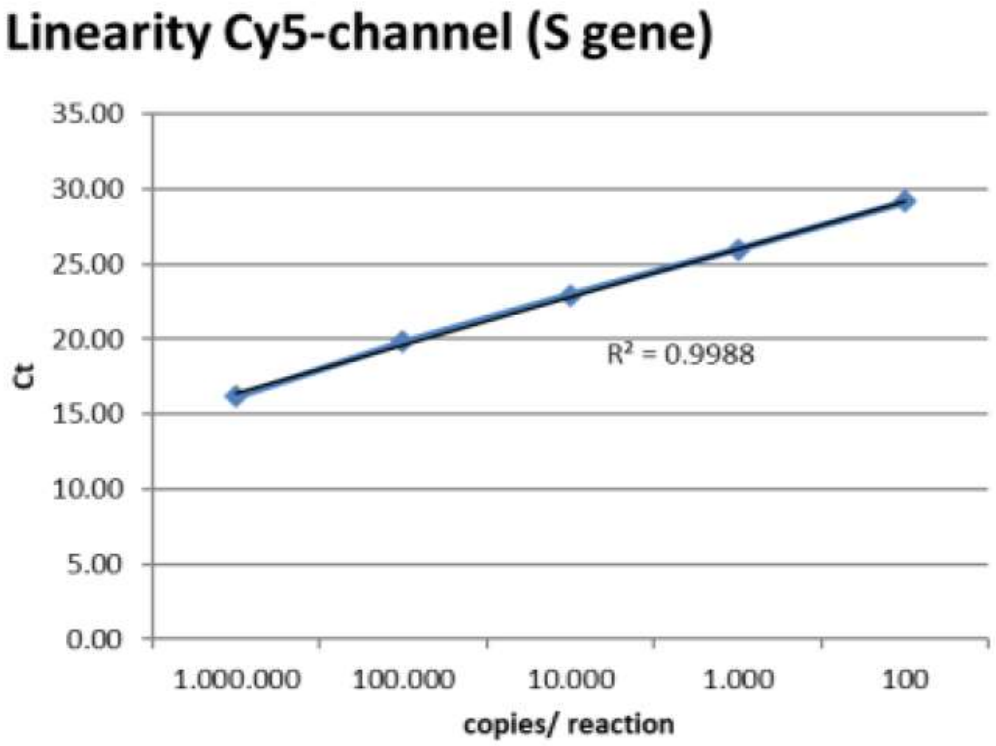


Figure 7: Determination of the linear range of MutaPLEX® SC2 multi gene Real-Time-RT-PCR in the Cy5 channel.

16.5 Precision

The precision of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit was determined as intra-assay variability, inter-assay variability and inter-lot variability.

Variability data are expressed by standard deviation and coefficient of variation. The data are based on quantification analyses of defined concentrations of RdRP gene (SARS-CoV-2) in vitro transcripts, E gene (SARS-CoV-2) in vitro transcripts, S gene (SARS-CoV-2) in vitro transcripts and on the threshold cycle of the Control RNA (IPC). The results are shown in table 10.

Table 10: Precision of the MutaPLEX® SC2 multi gene Real-Time-RT-PCR Kit.

RdRP gene (FAM)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	100	0.18	0.58
Inter-Assay Variability	100	0.70	2.25
Inter-Lot Variability	100	0.17	0.54

E gene (ROX)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	10	0.33	0.98
Inter-Assay Variability	10	0.90	2.76
Inter-Lot Variability	10	0.11	0.34

S gene (Cy5)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	10	0.15	0.47
Inter-Assay Variability	10	0.21	0.65
Inter-Lot Variability	10	0.18	0.56

IPC (HEX)	copies/ µl	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	1000	0.78	2.45
Inter-Assay-Variability	1000	1.13	3.57
Inter-Lot-Variability	1000	0.42	1.33

16.6 Diagnostic Sensitivity

The diagnostic sensitivity of Real-Time-RT-PCR assays is mainly dependent on the DNA/RNA extraction method used to isolate DNA and RNA from various biological specimens. DNA/RNA extraction reagents are not part of the Immundiagnostik AG Real-Time-RT-PCR kits. Immundiagnostik AG Real-Time-RT-PCR kits include an extraction control and guidelines for the validation criteria of the extraction control in each reaction. The extraction control indicates inhibition of the Real-Time-RT-PCR and/or inefficient nucleic acid extraction. It cannot be used as a calibrator.

Therefore, Immundiagnostik AG guarantees the analytical sensitivities and specificities of the real time RT- PCR kits, performed with eluted DNA and RNA from reference materials and ring trial samples and with synthetic nucleic acid fragments. Immundiagnostik AG does not guarantee diagnostic sensitivities. If diagnostic sensitivities are mentioned in manuals of Immundiagnostik AG Real-Time-RT-PCR kits, the data are strictly correlated to a specific nucleic acid extraction method that has been used during the validation of the respective kits and cannot be transferred to other extraction methods.

It is the responsibility of the user to qualify the extraction methods used for DNA/RNA isolation from biological samples.

17 ABBREVIATIONS AND SYMBOLS

(c)DNA	(complementary) Deoxyribonucleid acid	REF	Catalog number
RNA	Ribonucleid acid	→REF	To be used with
PCR	Polymerase chain reaction		Contains sufficient for <n> test
RT	Reverse transcription		Upper limit of temperature
RT-PCR	Reverse transcription-PCR		Manufacturer
REACTION MIX	Reaction mix		Use by
ENZYME	Enzyme	LOT	Lot number
CONTROL +	Positive control	CONT	Content
CONTROL –	Negative control		Consult instructions for use
CONTROL RNA IPC	Control RNA (IPC)	IVD	*In vitro* diagnostic medical device
RdRP gene	RNA-dependent RNA polymerase gene	S gene	Spike gene
E gene	Envelope gene		

18 LITERATURE

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3. Corman et al. Detection of 2019 novel coronavirus (2019-nCoV) by realtime RT-PCR. Eurosurveillance, Volume 25, Issue 3, 23/Jan/2020.
4. https://www.who.int/publications/i/item/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117
5. https://www.nhs.uk/conditions/sars/